

Figure 1. DNA and amino acid sequences of the mouse 21.6 light chain variable region.

```

      atgaggggcccctgctcagatttttggattcttggtcaggagacgttgtagaaatgagacc
1  -----+-----+-----+-----+-----+-----+-----+-----+ 60
      tactccccggggacgagtcctaaaaacctaagaaccagtcctctgcaacatctttactctgg
ACTAGTCGACATGAGGGCCCCCTGCTCAGTTTTTTGGCTTCTTG-3'
      A          A C    AA
      MKV4 Primer
      gtctattcagttcctggggctcttgttgttctggcttcatgggtgctcagtgtagacatcca
161 -----+-----+-----+-----+-----+-----+-----+-----+ 120
      cagataagtcaaggacccccgagaacaacaagaccgaagtaccacgagtcacactgtaggt
      S I Q F L G L L L F W L H G A Q C][D I Q -
      Leader
      gatgacacagtcctccatcctcactgtctgcatctctgggaggcaaagtcaccatcacttg
121 -----+-----+-----+-----+-----+-----+-----+-----+ 180
      ctactgtgtcagaggtaggagtgacagacgtagagaccctccgtttcagtggttagtgaac
      M T Q S P S S L S A S L G G K V T I T C] -
      FR1
      caagacaagccaagacattaacaagtatatggcttggtaccaacacaagcctggaaaacg
181 -----+-----+-----+-----+-----+-----+-----+-----+ 240
      gttctgttcggttctgtaattgttcatataccgaaccatgggttggttcggaccttttgc
      [K T S Q D I N K Y M A][W Y Q H K P G K R -
      CDR1          FR2
      tcctaggctgctcatacattacacatctgcattacagccaggcatcccatcaagggttcag
241 -----+-----+-----+-----+-----+-----+-----+-----+ 300
      aggatccgacgagtatgtaattgtgtagacgtaattgtcgggtccgtagggtagttccaagtc
      P R L L I H][Y T S A L Q P][G I P S R F S -
      CDR2
      tggaagtgggtctgggagagattattccttcaacatcagcaacctggagcctgaagatat
301 -----+-----+-----+-----+-----+-----+-----+-----+ 360
      accttcacccagaccctctctaataaggaagttgtagtcggttggaacctcggacttctata
      G S G S G R D Y S F N I S N L E P E D I -
      FR3
      tgcaacttattattgtctacagtatgataatctgtggacgttcgggtggaggcaccaagct
361 -----+-----+-----+-----+-----+-----+-----+-----+ 420
      acgttgaataataacagatgtcatactattagacacctgcaagccacctccgtgggttcga
      A T Y Y C][L Q Y D N L W T][F G G G T K L -
      CDR3          FR4
      Mouse Kappa Primer
      3'-GTAGAAGGGTGGTAGGTGGGCCCT
      ggaaatcaaacgggctgatgctgcaccaactgtatccatcttcccaccatccacccggga
421 -----+-----+-----+-----+-----+-----+-----+-----+ 480
      ccttttagtttgcccgaactacgacgtgggttgacataggtagaagggtggtaggtgggcccct
      E I K]
      AGG-5'
      tcc
481 --- 483
      agg

```

Figure 2. DNA and amino acid sequences of the mouse 21.6 heavy chain variable region.

```

      atgaaatgcagctgggtcatgttcttctgatggcagtggttacaggggtcaattcagag
1  -----+-----+-----+-----+-----+-----+-----+ 60
      tactttacgtcgacccagtcacaagaaggactaccgtcaccaatgtccccagttaagtctc
ACTAGTCGACATGAAATGCAGCTGGGTCATCTTCTTC-3'
                                G
      MHV1 Primer
      [M K C S W V M F F L M A V V T G V N S][E -
                                Leader

      gttcagctgcagcagctctggggcagagcttgtgaagccaggggcctcagtcgaagttgtcc
61 -----+-----+-----+-----+-----+-----+ 120
      caagtcgacgtcgtcagaccccgctctcgaacacttcgggtccccggagtcagttcaacagg
      V Q L Q Q S G A E L V K P G A S V K L S -
                                FR1

      tgcacagcttcttggttcaacattaaagacacctatatacactgtgtgaagcagaggcct
121 -----+-----+-----+-----+-----+-----+ 180
      acgtgtcgaagaccgaagttgtaatttctgtggatatatgtgacacacttcgtctccgga
      C T A S G F N I K][D T Y I H][C V K Q R P -
                                CDR1

      gaacagggcctggagtggttgaaggattgatcctgcgaatgggtataactaaatatgac
181 -----+-----+-----+-----+-----+-----+ 240
      cttgtccccggacctcacctaaccttcctaactaggacgcttaccaatatgatttatactg
      E Q G L E W I G][R I D P A N G Y T K Y D -
                                FR2                                CDR2

      ccgaagttccagggcaaggccactataacagctgacacatcctccaacacagcctacctg
241 -----+-----+-----+-----+-----+-----+ 300
      ggcttcaaggtcccggtccggtgatattgtcgactgtgtaggaggttgtgtcggatggac
      P K F Q G][K A T I T A D T S S N T A Y L -

      cagctcagcagcctgacatctgaggacactgccgtctatttctgtgctagagagggatat
301 -----+-----+-----+-----+-----+-----+ 360
      gtcgagtcgtcggactgtagactcctgtgacggcagataaagacacgatctctccctata
      Q L S S L T S E D T A V Y F C A R][E G Y -
                                FR3

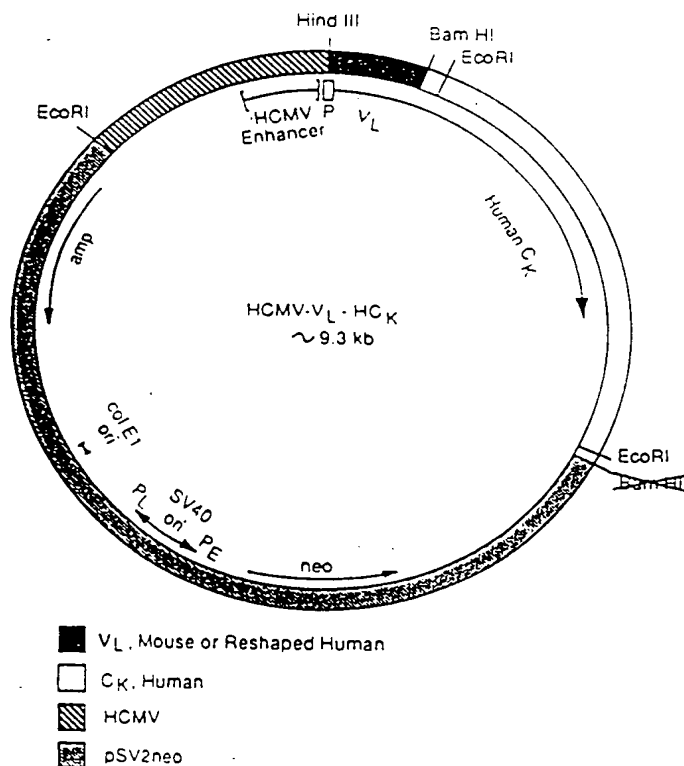
      tatggtaactacggggtctatgctatggactactgggggtcaaggaacctcagtcaccgctc
361 -----+-----+-----+-----+-----+-----+ 420
      ataccattgatgccccagatacgtacgtgatgaccccagttccttggagtcagtgggcag
      Y G N Y G V Y A M D Y][W G Q G T S V T V -
                                CDR3                                FR4

                                Mouse Gamma-1 Primer
                                3'-GTAGACAGATAGGTGACCGGGCCCTAGG-5'
      tcctcagccaaaacgacacccccatctgtctatccactggccccgggatcc
421 -----+-----+-----+-----+-----+-----+ 470
      aggagtcgggttttgcgtgtgggggtagacagataggtgaccggggccctagg
      S S]

```

**Figure 3. Mammalian cell expression vectors used to produce chimeric and reshaped human antibodies with human kappa light chains and human gamma-1 heavy chains.**

**A. Light chain expression vector.**



**B. Heavy chain expression vector.**

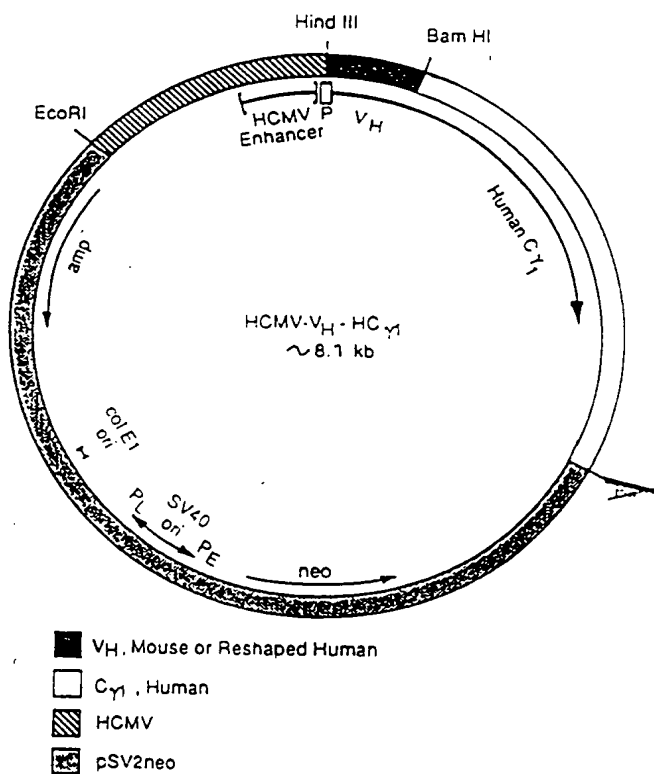


Figure 4. ELISA testing the ability of chimeric 21.6 antibody to bind to L cells expressing human  $\alpha 4\beta 1$  integrin on their surface .

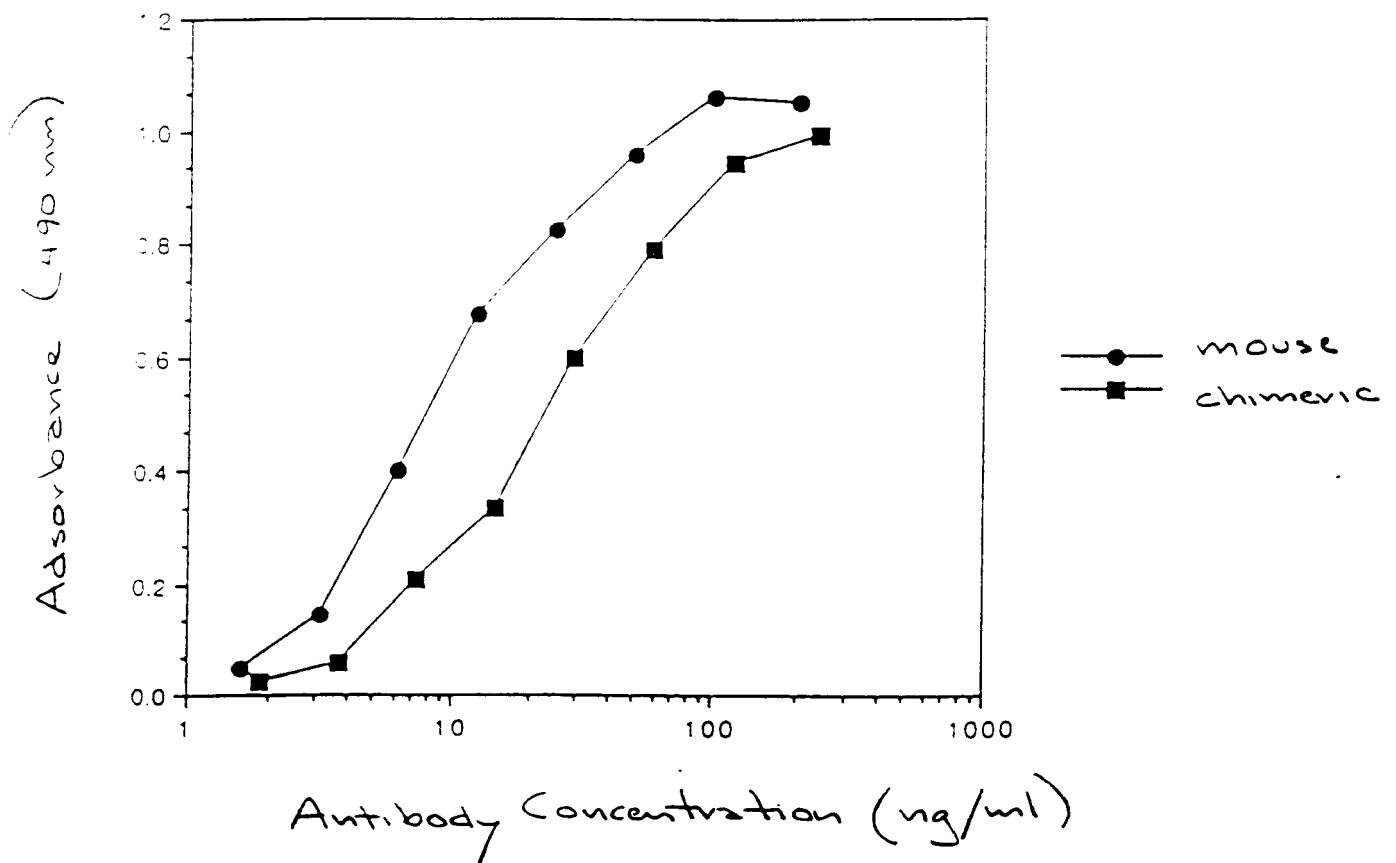


Figure 5. A view of the molecular model of the variable regions of mouse 21.6 antibody.

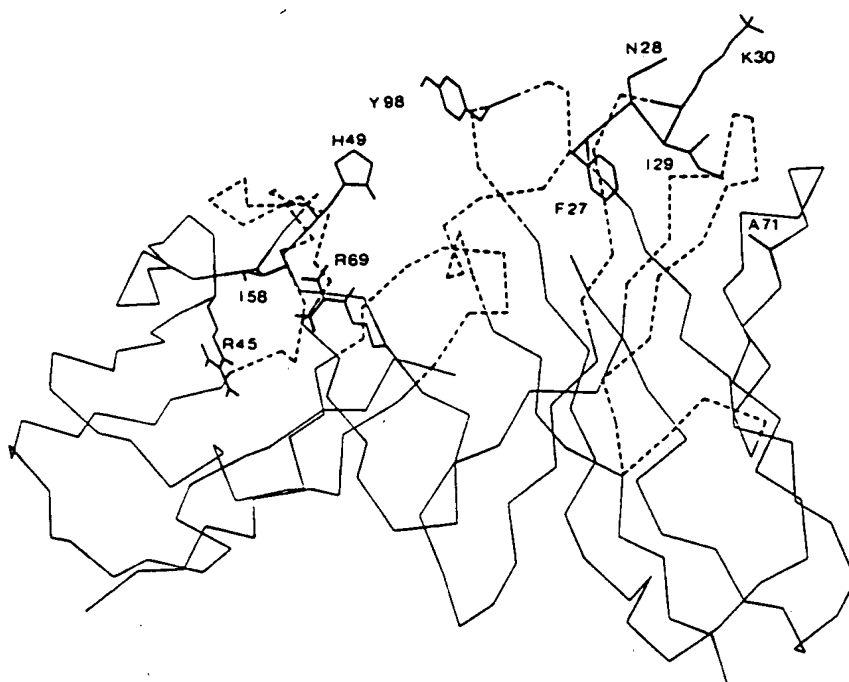


Figure 6. Comparisons of the amino acid sequences of mouse and reshaped human 21.6 light chain variable regions.

	FR1 1	2	CDR1 3	FR2 4	CDR2 5
	12345678901234567890123		45678901234	567890123456789	0123456
	*		*****		*
21.6	DIQMTQSPSSLSASLGGKVTITC		KTSQDINKYMA	WYQHKPGKRPRLLIH	YTSALQP
REI	DIQMTQSPSSLSASVGDRTITC		QASQDIISKYLN	WYQQTPGKAPKLLIY	EASNLQA
La	DIQMTQSPSSLSASVGDRTITC		KTSQDINKYMA	WYQQTPGKAPRLLIH	YTSALQP
Lb	-----		-----	-----R----	-----

	FR3 6	7	8	CDR3 9	FR4 10
	78901234567890123456789012345678			901234567	8901234567
	*			*****	
21.6	GIPSRFSGSGSGRDYSFNISNLEPEDIATYYC			LQYDNL-WT	FGGGTKLEIK
REI	GVPSRFSGSGSGTDYFTFTISSLQPEDIATYYC			QQYQSLPYT	FGQGTKLQIT
La	GIPSRFSGSGSGRDYFTFTISSLQPEDIATYYC			LQYDNL-WT	FGQGTKVEIK
Lb	-I-----			-----R-----	-----VE-K

Figure 7. Comparisons of the amino acid sequences of the mouse and reshaped human 21.6 heavy chain variable regions.

	FR1										CDR1					FR2					CDR2																																													
	1		2		3		4		5		6		7		8		9		10																																															
21.6	E	V	Q	L	Q	S	G	A	E	L	V	K	P	G	A	S	V	K	L	S	C	T	A	S	G	F	N	I	K	D	T	Y	I	H	C	V	K	Q	R	P	E	Q	G	L	E	W	I	G	R	I	D	P	A	N	G	Y	T	K	Y	D	P	K	F	Q	G	
2*CL	Q	V	Q	L	V	Q	S	G	A	E	V	K	K	P	G	A	S	V	K	V	S	C	K	A	S	G	Y	T	F	S	Y	A	M	H	W	V	R	Q	A	P	G	Q	R	L	E	W	M	G	W	I	N	A	G	N	G	N	T	K	Y	S	Q	K	F	Q	G	
Ha	Q	V	Q	L	V	Q	S	G	A	E	V	K	K	P	G	A	S	V	K	V	S	C	K	A	S	G	F	N	I	K	D	T	Y	I	H	W	V	R	Q	A	P	G	Q	R	L	E	W	M	G	R	I	D	P	A	N	G	Y	T	K	Y	D	P	K	F	Q	G
Hb	-----F										N	I	K	-----G					-----																																															
Hc	-----F										N	I	K	-----					-----																																															

	FR3										CDR3					FR4																																								
	7		8		9		10		11		12		13		14																																									
21.6	K	A	T	I	A	D	T	S	S	N	T	A	Y	L	Q	L	S	S	L	T	S	E	D	T	A	V	Y	F	C	A	R	E	G	Y	Y	G	N	Y	G	V	Y	A	M	D	Y	W	G	Q	G	T	S	V	T	V	S	
2*CL	R	V	T	I	T	R	D	T	S	A	S	T	A	Y	M	E	L	S	S	L	R	S	E	D	T	A	V	Y	Y	C	A	R	G	G	Y	Y	G	S	G	S	----	N	Y	W	G	Q	G	T	L	V	T	V	S			
Ha	R	V	T	I	T	R	D	T	S	A	S	T	A	Y	M	E	L	S	S	L	R	S	E	D	T	A	V	Y	Y	C	A	R	E	G	Y	Y	G	N	Y	G	V	Y	A	M	D	Y	W	G	Q	G	T	L	V	T	V	S
Hb	-----A										-----					-----																																								
Hc	-----A										-----F					-----																																								

Figure 8. Diagram of the PCR-based construction of version "a" of reshaped human 21.6 light chain variable region.

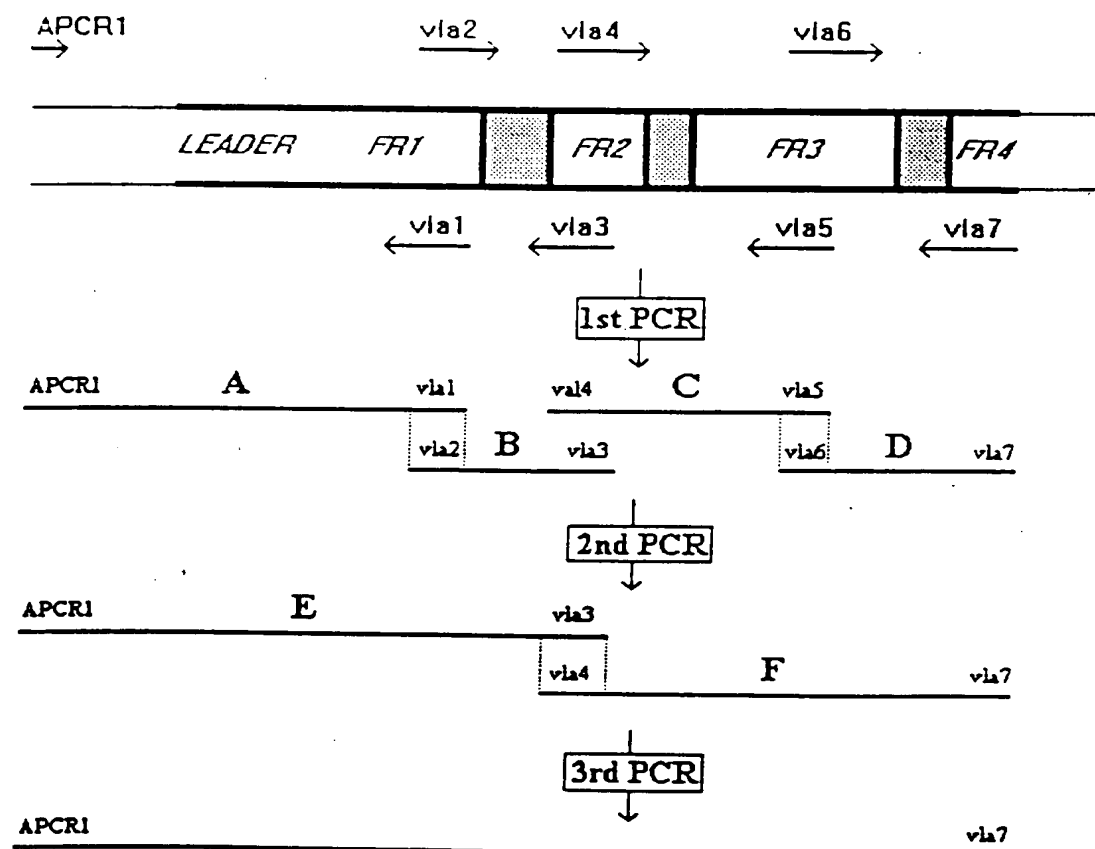




Figure 9. Diagram of the PCR-based construction of version "a" of reshaped human 21.6 heavy chain variable region.

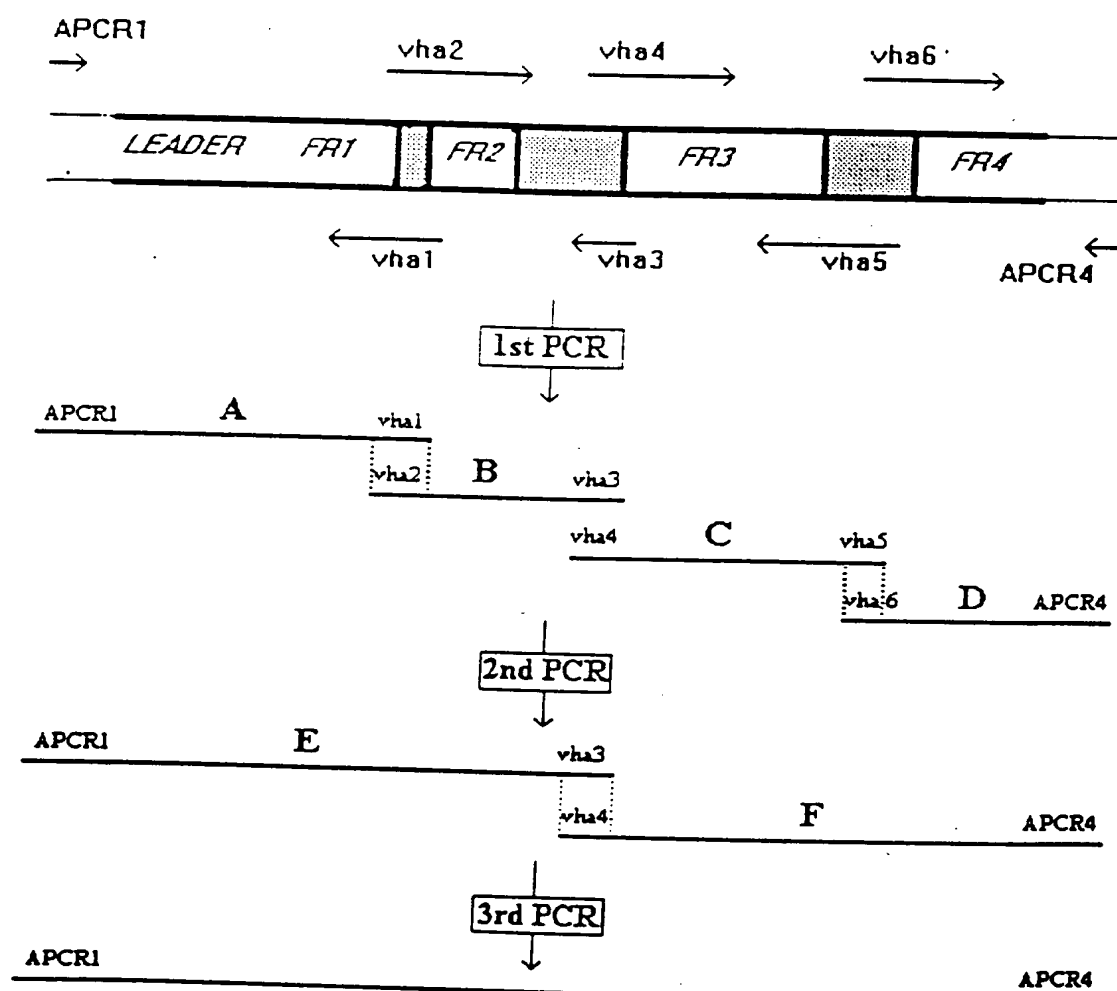


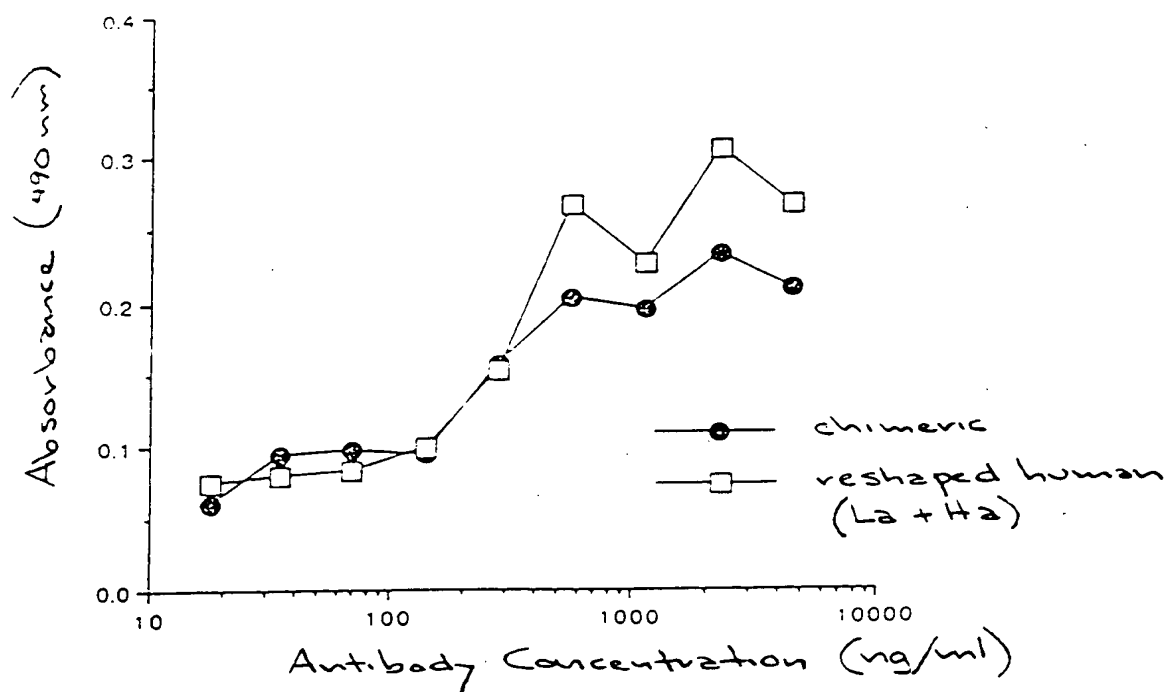
Figure 10. DNA and amino acid sequences of the first version ("a") of reshaped human 21.6 light chain variable region.

HindIII Kozak sequence  
 1 aagcttgcgcccaccatgagaccgtctattcagttcctggggctcttggtgttctggctt 60  
 -----+-----+-----+-----+-----+  
 ttcgaacggcggtggtactctggcagataagtcaaggaccccgagaacaacaagaccgaa  
 [M R P S I Q F L G L L L F W L -  
 Leader  
 catggtgctcagtggtgacatccagatgacacagtcctccatcctcactgtctgcatctGTA  
 61 -----+-----+-----+-----+-----+ 120  
 gtaccacgagtcacactgtaggtctactgtgtcagaggtaggagtgacagacgtagaCAT  
 H G A Q C][D I Q M T Q S P S S L S A S V -  
 FR1  
 ggaGATAGAgtcaccatcacttgcaagacaagccaagacattaacaagtatatggcttgg  
 121 -----+-----+-----+-----+-----+ 180  
 cctCTATCTcagtggttagtgaacgttctgttcggttctgttaattgttcataataccgaacc  
 G D R V T I T C][K T S Q D I N K Y M A][W -  
 CDR1  
 taccaaCAGACAcctggaaaaGCTcctaggctgctcatatacattacacatctgcattacag  
 181 -----+-----+-----+-----+-----+ 240  
 atgggttGTCTGTggaccttttCGAggatccgacgagtagtgaatgtgtgtagacgtaatgtc  
 Y Q Q T P G K A P R L L I H][Y T S A L Q -  
 FR2 CDR2  
 ccaggcatcccatcaagggttcagtggaagtgggtctgggagagattatACTttcACCatc  
 241 -----+-----+-----+-----+-----+ 300  
 ggtccgtagggttagttccaagtcaccttcacccagaccctctctaataTGAaagTGGtag  
 P][G I P S R F S G S G S G R D Y T F T I -  
 FR3  
 agcAGCctgCAGcctgaagatattgcaacttattattgtctacagtatgataatctgtgg  
 301 -----+-----+-----+-----+-----+ 360  
 tcgTCGgacGTCggacttctataacgttgaataataacagatgtcatactattagacacc  
 S S L Q P E D I A T Y Y C][L Q Y D N L W -  
 CDR3  
 acgttcggtCAAggcaccaagGTGgaaatcaaacgtgagtggtatcc  
 361 -----+-----+-----+-----+-----+ 406  
 tgcaagccaGTTccgtggttcCACcttttagtttgcaactcacctagg  
 T][F G Q G T K V E I K]  
 FR4

**Figure 11. DNA and amino acid sequences of the first version ("a") of reshaped human 21.6 heavy chain variable region.**

Figure 12. ELISA testing the ability of chimeric and reshaped human 21.6 antibodies to bind to L cells expressing human  $\alpha 4\beta 1$  integrin on their surface.

Panel A. Analysis of reshaped human 21.6 antibody (La + Ha).



Panel B. Analysis of reshaped human 21.6 antibody (La + Hc).

